Vitamin D deficiency down-regulates Notch pathway contributing to skeletal muscle atrophy in old Wistar rats

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INTRODUCTION: **The aged-related decrease in skeletal muscle regeneration capacity is a contributing factor to sarcopenia [1]. Muscle regeneration depends in part on satellite cells whose reserve pool is reduced during aging [1]. The decreased activity of the Notch signalling pathway with aging likely explains the reduction in the proliferation potential of satellite cells, as this pathway is involved in self-renewal of these cells [2]. Skeletal muscle is a primary target of vitamin D action. It modulates muscle proliferation and differentiation *in vitro* and stimulates muscle regeneration *in vivo* [3-4]. The vitamin D status is positively correlated with skeletal muscle mass and function. Hypovitaminosis D frequently observed in the elderly is responsible for muscle weakness [5-6].**

AIM OF THE STUDY: Evaluation of how vitamin D deficiency induces skeletal muscle atrophy in old rats through a reduction in Notch pathway activity and proliferation potential in muscle.

EXPERIMENTAL PROTOCOL:

15-month-old Male Wistar Rats (n=20)

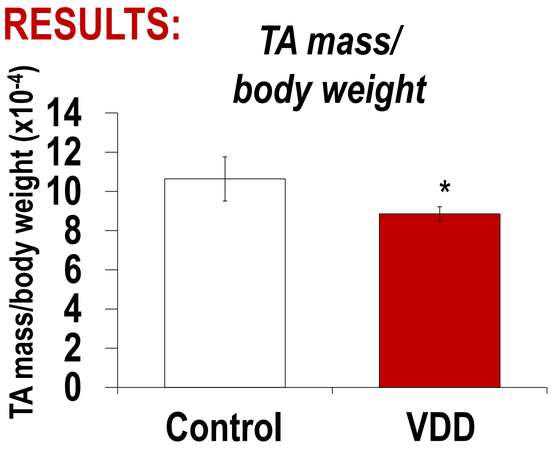
Standard Diet → **24-month-old Male Wistar Rats** → **control group** (n=10)

9 months

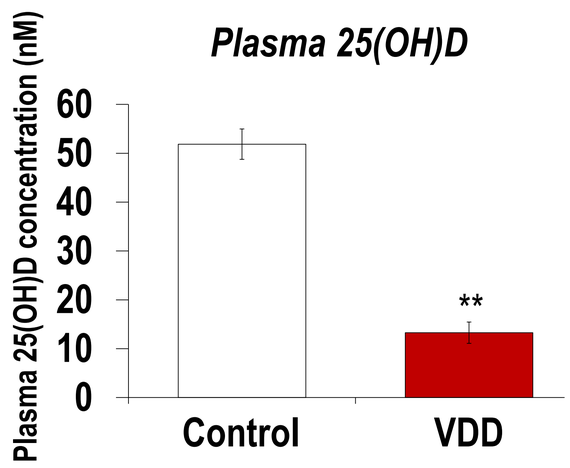
Vitamin D-depleted Diet → **vitamin D-depleted (VDD) group** (n=10)

ELISA for circulating 25(OH)D measurment, qPCR for gene expressions and western-blot for protein expressions in *tibialis anterior (TA)* muscle

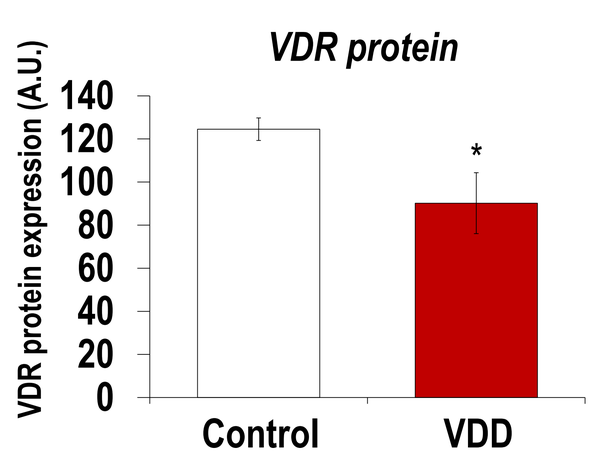
RESULTS:



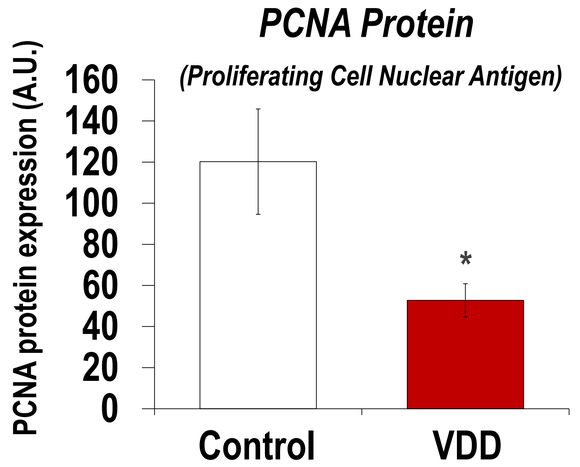
Vitamin D deficiency leads to TA mass decrease.



Plasma 25(OH)D concentration is reduced in VDD rats.

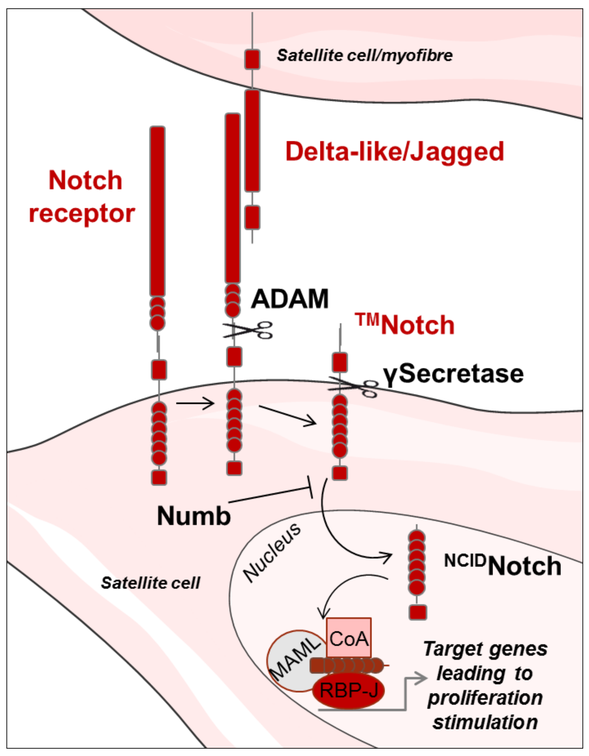


Vitamin D receptor (VDR) expression is diminished in TA of VDD rats.

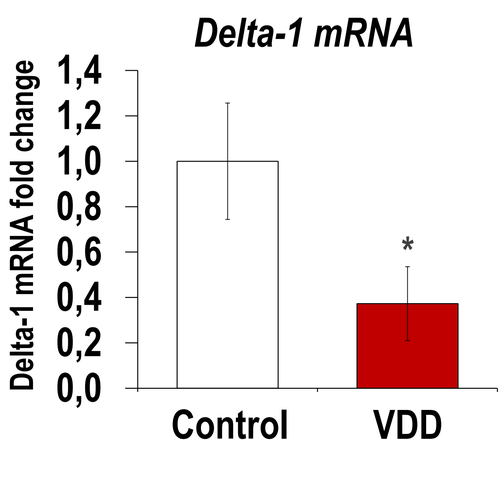


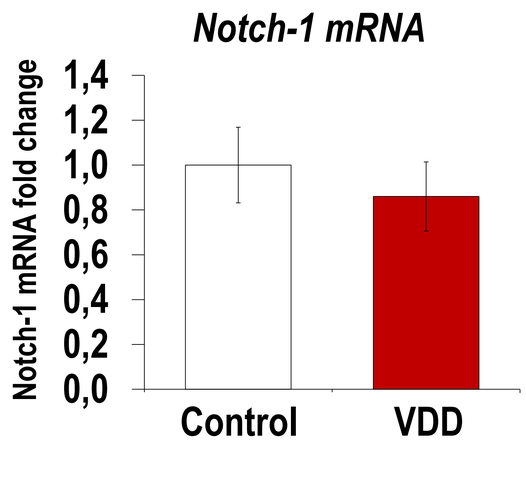
The expression of the proliferation marker PCNA is reduced in TA of VDD rats.

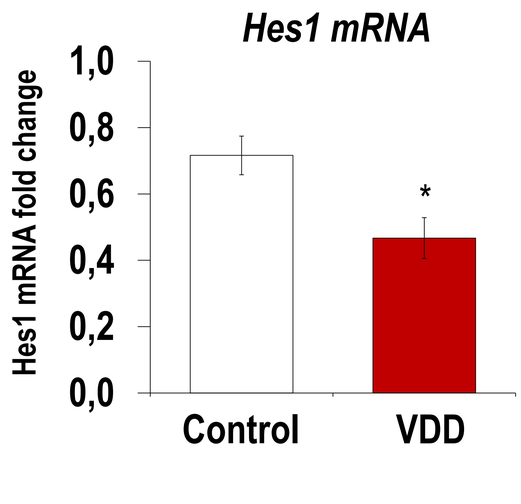
Notch signalling pathway

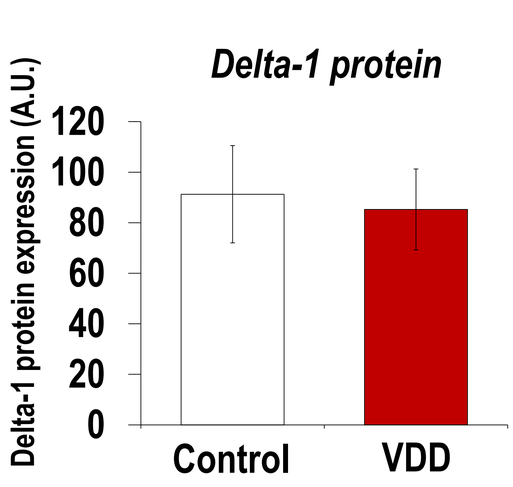


After injury, Delta-like is stimulated in satellite cells and myofibers. Delta-like binds to Notch receptor which activates Notch pathway. Then two proteolytic cleavage events occur. The first involves an ADAM protease (a Disintegrin and Metalloprotease) cleaving the Notch receptor and generating a transmembrane fragment of Notch: TMNotch. TMNotch is then cleaved by a γ-secretase complex, leading to the release of the intracellular domain of Notch: NicdNotch. NicdNotch migrates to the nucleus where it acts as a transcription factor, stimulating the expression of target genes as Hes1. These genes are involved in the inhibition of differentiation and the maintenance of cell renewal.

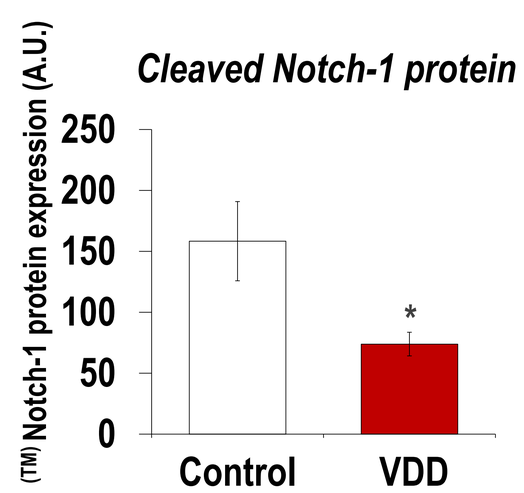








The gene expression of Delta1 decreases in TA of VDD old rats but the protein expression is not modified.



The gene expression of Notch-1 is not changed following vitamin D depletion. The protein expression of cleaved Notch form is reduced in TA of VDD old rats.

The gene expression of the target of Notch-1, Hes1, is reduced in TA of VDD old rats.

Notch pathway activity is diminished in TA muscle of VDD old rats.

Mean ± SEM ; Statistics : T-test of Student, * p< 0,05 ou ** p< 0,01 *versus* control group.

CONCLUSION : **Vitamin D depletion for 9 months efficiently induced vitamin D deficiency in old rats. Vitamin D depletion induces skeletal muscle atrophy in old rats through a reduction in the proliferative ability and in Notch pathway activity in skeletal muscle. In this context, vitamin D deficiency could further aggravate the age-related impaired capacity of muscle to regenerate.**

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[1] Barberi (2013), [2] Conboy (2003), [3] Ceglia (2009), [4] Stratos (2013), [5] Wacker (2013), [6] Holick (2006)